E-21-F4a

FINAL REPORT

BRP Project Prime # ES10846: Sample Preparation for Genomic Analysis in Micro Device Format

Specific Aims: The overall goal of the proposed research is to produce an integrated sample preparation micro-analytical device for preparing blood samples for genomic analyses. The strategy is to develop a front-end micro sample preparation system, μ-SPS, for use as a research tool with the flexibility to be integrated with a number of downstream genetic analysis platforms, i.e. either sequencing or genotyping. The μ-SPS is composed of three main micro-compartments including: 1.) Sample introduction, combined with cell sorting and selection. 2.) Cell lysis, recovery of the nucleic acid material of choice (e.g. DNA or mRNA), and sample clean up via solid phase extraction or affinity capture. 3.) Elution of the material to an amplification μ-compartment, and subsequent amplification (e.g. via PCR or rtPCR). In years 1-3 of the grant, the primary focus of the grant was development of the functional microcompartments (e.g., microcompartments for white blood cell collection, cell lysis, DNA extraction, PCR, electrophoresis) needed for the total analysis system while keeping in mind the future need for integration of the functional compartments. In years 3-5 of the grant, studies of the μ-compartment prototypes were paralleled by an investigation of techniques for integrating the μ-compartments into a monolithic and hybrid μ-SPS. In funding year 5, the BRP group provided solutions for integrating multiple functional compartments into a multi-functional system. The integration solutions included the development of micro-to-macro interface technology for complex microfluidic systems, methods of integrating actuation components (i.e. pumps and valves) into complex, multi-functional microfluidic systems, methods for simultaneously integrating electrical detection systems (e.g., conductivity detectors, impedance spectroscopy) and optical detection systems (e.g., resonance light scattering, fluorescence based technologies), and methods for creating the monolithic, multi-compartment microfluidic systems.

The specific aims for this project have remained unmodified over the project duration. When the project began six years ago, there were not any demonstrations of complex microfluidic based analysis systems with multiple functional compartments and packaging solutions for these complex microsystems. At best, laboratories around the world were working on the development of individual functional micro analysis systems including solutions for PCR, electrophoresis, immunoassay, etc. This project represented a marriage of cutting edge science and futuristic engineering technologies.

The following section and the attached publications are focused the activities in the final 12-24 months of the project. By this time in the project, most of the functional compartments had been realized and the performance of the functional compartments improved to meet or exceed the requirements of the μ-SPS.

Studies and Results:

Aim 1: Sample Introduction, Cell Sorting, Selection and Lysis: One of the toughest challenges for the BRP project was the development of the microfluidic system with the capability to purifying white blood cells from the initial unmodified blood sample. In years 3-5, the Frazier laboratory developed a microsystem for separating red and white blood cells (WBC) from whole blood samples. The microsystem was used as the first compartment of the completed μ-SPS to extract the white blood cells and subsequently the DNA. For this project, the red blood cells (RBC) and other blood components are routed to a waste reservoir. The Frazier laboratory demonstrated microsystems using both paramagnetic and diamagnetic forces as the basis for the blood separation with 98+% efficiencies. Details of the blood cell separation technologies can be found in the attached journal reprints.

Aim 4: Integration of Independent Prototype Micro Compartments into a Hybrid μ-SPS: A microchip design for fully integrated (SPE-PCR-CE) processing was designed, with the valve locations and reservoirs required to allow continuous processing incorporated. Glass devices were then fabricated according to this design in the Frazier laboratory, and polymeric interfaces were fabricated to provide the valves and reservoir connections. The interfaces were then secured to the glass microfluidic system. These devices were tested in the Landers laboratory to see if the valves functioned as required and if the interfaces performed as needed during device operation (e.g., PCR heat cycling, sol-gel loading for DNA extraction). Using a valve in the SPE region, we were able to pack the SPE chamber with beads and glue them in place using the sol-gel material. Furthermore, we were able to perform extraction using this chip with the integrated interface. The attached journal reprints show the results of the interface development and the performance of the μ-SPS system using the integrated interface.

Significance: The BRP team has made enabling contributions to the field of micro total analysis systems. The project has offered innovative, creative solutions to the difficult problems associated with handling complex biological samples in microfluidic systems as well as solutions for developing complex multi-functional micro total analysis systems including integration and packaging.

Publications

Over the lifetime of the grant, the BRP partnership has produced in excess of 111 journal publications and 57 prestigious conference publications. The publications for year 5 of the grant are listed first, followed by a complete list of publications from Years 1-4.

Year 5:

1. M. Graff, S.K. Mohanty, E. Moss, and A.B. Frazier, "Microstenciling: A Generic Technology for Microscale Patterning of Vapor Deposited Materials," *Journal of Microelectromechanical Systems,* **13**(6), 956-962 (2004).
2. K.H. Han and A.B. Frazier, "Continuous Magnetophoretic Seperation of Blood Cells in Micro Device Format", *Journal of Applied Physics*, (96) 5797-5802 (2004).
3. K.H. Han and A.B. Frazier, "Continuous Magnetophoretic Separation of Blood Cells in Micro Device Format", *Virtual Journal of Nanoscale Science & Technology*, (10) 5797-5802 (2004). Invitation Only
4. K.H. Han and A.B. Frazier, "Continuous Magnetophoretic Separation of Blood Cells in Micro Device Format", *Virtual Journal of Biological Physics Research*, (8) 5797-5802 (2004). Invitation Only
5. A. Han, M. Graff, O. Wang, and A.B. Frazier, "An Approach to Multi-Layer Microfluidic Systems with Integrated Electrical, Optical, and Mechanical Functionality", *IEEE Sensors Journal*, 5(1) 82-89 (2005).
6. A. Han, K.H. Han, S. Mohanty, and A.B. Frazier, "Microsystems for Whole Blood Purification and Electrophysiological Analysis", *Journal of Semiconductor Technology and Science*, (5) 1-10 (2005).
7. K.H. Han and A.B. Frazier, "Reliability Aspects of Packaging and Integration Technology for Microfluidic Systems", *Transaction on Device and Materials Reliability*, 5(3) 452-457 (2005).
8. K.H. Han and A.B. Frazier, "Diamagnetic Capture Mode Magnetophoretic Microseparator for Blood Cells Using Native Magnetic Properties", *Journal on Microelectromechanical Systems*, 14 (6) 1422-1431 (2005).
9. K.H. Han and A.B. Frazier, "Paramagnetic Capture Mode Cascade Magnetophoretic Microseparator for High Efficiency Blood Cell Separations", *Lab-on-a-Chip*, 6 (2) 265–273 (2006).
10. M. Graff and A.B. Frazier, "Resonance Light Scattering (RLS) Detection of NanoParticle Separations in a Micro Electrical Field-Flow Fractionation System", *IEEE Journal on Nanotechnology*, 5 (1) 8-13 (2006).
11. K.H. Han, A. Han and A.B. Frazier, "Microsystems for Collection and Electrophysiological Analysis of Circulating Breast Cancer Cells", *Biosensors and Bioelectronics*, 21(10) 1907-1914 (2006).
12. K.H. Han and A.B. Frazier, "Paramagnetic Capture Mode Magnetophoretic Microseparator for Blood Cells", *IEE Proceedings on Nanobiotechnology*, 153 (4) 67-73 (2006).
13. A. Han and A.B. Frazier, "Ion Channel Characterization using Single Cell Impedance Spectroscopy", *Lab-on-a-Chip*, 6 (11) 1412-1414 (2006).
14. E.D. Moss, A. Han, and A.B. Frazier, "A Fabrication Technology for Multi-layer Polymer-based Microsystems with Integrated Fluidic and Electrical Functionality", *Sensors and Actuators B*, Date Accepted: April 28, 2006.
15. K.H. Han, R. McConnell, C. Easley, J. Bienvenue, J. Ferrance, J. Landers, and A.B. Frazier, "An Active Microfluidic System Interface Technology", *Sensors and Actuators B*, Date Accepted: June 21, 2006, doi:10.1016/j.snb.2006.06.028.
16. A. Han and A.B. Frazier, "Quantification of the Heterogeneity in Breast Cancer Cell Lines Using Whole Cell Impedance Spectroscopy", *Clinical Cancer Research*, Date Accepted: September 9, 2006.
17. K. Han, R. McConnell, J. Ferrance, J. Landers, and A.B. Frazier, "Integrated Interface Technology for Microfluidic Systems", *Solid State Sensor and Actuator Workshop*, Hilton Head, SC, June 2004, pp. 108-112.
18. K. Han and A.B. Frazier, "Paramagnetic Capture Mode Magnetophoretic Microseparator for Blood Cells", *State Sensor and Actuator Workshop*, Hilton Head, SC, June 2004, pp. 248-249.

19. T. Edwards and A.B. Frazier, "An Acoustic Field Flow Fractionation System for Nano Scale Separations", *International Conference on Micro Total Analysis Systems*, Netherlands, October 2004.

20. M. Graff, T.L. Edwards, B.K. Gale, and A.B. Frazier, "Nanoparticle Separations Using Miniaturized Field-Flow Fractionation Systems", *NSTI Nanotechnology Conference*, May 2005.

21. K.H. Han and A.B. Frazier, "A Microfluidic System for Continuous Magnetophoretic Separation of Suspended Cells Using Their Native Magnetic Properties", *NSTI Nanotechnology Conference*, May 2005.

22. A. Han, E. Moss, and A.B. Frazier, "Whole Cell Electrical Impedance Spectroscopy for Studying Ion Channel Activity", *13th International Conference on Solid-State Sensors and Actuators (Transducers05)*, Seoul, Korea, June 2005, pp. 1704-1707.

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Department of Health and Human Services

Final Invention Statement and Certification

(For Grant or Award)

DHHS Grant or Award No.: ES10846

A. We hereby certify that, to the best of our knowledge and belief, all inventions are listed below which were conceived and/or first actually reduced to practice during the course of work under the above-referenced DHHS grant or award for the period

05/01/00 *original effective date* through 3/31/06 *date of termination*

B. Inventions (Note: If no inventions have been made under the grant or award, insert the word *"NONE"* under Title below.)

NAME OF INVENTOR	TITLE OF INVENTION	DATE REPORTED TO DHHS
A.B. Frazier and KiHo Han	Blood Separations Systems in MicroDevice Format and Fabrication Methods (provisional patent)	10/20/2006
See Attached *(Use continuation sheet if necessary)*		

C. First Signature —The person responsible for the grant or award is required to sign (in ink). Sign in the block opposite the applicable type of grant or award.

TYPE OF GRANT OR AWARD	WHO MUST SIGN *(title)*	SIGNATURE
Research Grant	Principal Investigator or Project Director A. Bruno Frazier	
Health Services Grant	Director	
Research Career Program Award	Awardee	
All other types (*specify*):	Responsible Official	

D. Second Signature — This block ***must*** be signed by an official authorized to sign on behalf of the institution.

Title	Name and Mailing Address of Institution
Typed Name	
Signature / Date	

HHS 568 (Rev. 9/2004)

As reported from the James P. Landers laboratory, Department of Chemistry, University of Virginia, there were not any inventions, invention disclosures, patent applications, or patents as a result of this five year NIH grant.

As reported from the Robert Austin laboratory, Department of Physics, Princeton University, there were not any inventions, invention disclosures, patent applications, or patents as a result of this five year NIH grant.